

a. The above-identified patent application including pending claims 1-36;

b. The Office Action mailed by the United States Patent and Trademark Office on April 26, 1995;

c. Anderson and Sekelj, "Light-Absorbing and Scattering Properties of Non-Haemolysed Blood," *Phys. Med. Biol.*, 12(2):173-184, 1967;

d. Brown et al., U.S. Patent No. 4,134,678;

e. Curtis, U.S. Patent No. 5,064,282.

3. It is my understanding that the Examiner continues to reject claims of the above-identified patent application based upon the teachings of Anderson et al., and now rejects claims of the application based upon the teachings of Anderson et al. in combination with Brown et al.. I also understand that the Examiner relies upon the disclosure of Curtis to reject claims in this application. In my opinion, as presented in more detail below, none of these rejections is well-founded.

4. Turning first to the Anderson et al. reference, disclosed is that the difference in total optical attenuation before and after hemolysis of a particular suspension of red blood cells in isotonic saline is an estimate of the magnitude of light scattering before that particular blood cell suspension was hemolyzed. However, as shown in curve C in Figure 6 of Anderson et al., the magnitude of light scattering varies with total hemoglobin concentration. In a practical application, the hemoglobin concentration of a sample under test is completely unknown. Thus,

the only way to use Anderson et al. to "correct" for the effects of scattering, as suggested by the Examiner (see, for example, Office Action of 4/26/95 at page 6), is to first measure the total hemoglobin concentration of a sample under test by some independent method. This prior measurement would thus render any sort of measurement by the apparatus disclosed in Anderson et al. completely superfluous, and thus useless. In addition, this prior measurement would render the sample of "known" composition -- contrary to a requirement of the present invention that measurements be made on whole unaltered blood of unknown composition.

5. Even if one were to measure total hemoglobin concentration of each unknown sample by some independent method, as would be required to apply Anderson et al. to a practical application, the Examiner's "correction" still would not work, as the examples in the following paragraphs show.

6. First, Anderson et al. use non-hemolyzed red blood cells suspended in saline when conducting their observations. In particular, Anderson et al. state in the first full paragraph on page 177 that "fully oxygenated non-haemolyzed red cells suspended in isotonic saline were studied." Moreover, each of the graphs depicted in the Anderson et al. article, with the exception of Figure 5, expressly state that what was being studied were "red-cell suspensions," and it is quite likely that the measurements depicted in Figure 5 were also taken from red-cell suspensions. In addition, only oxygenated blood is being considered, which is but

one species of hemoglobin existing in unaltered whole blood. Thus Anderson et al. measure only altered blood of known composition, in contrast to the invention of the above-identified application.

7. The light scattering characteristics of red blood cells suspended in saline would be completely different from those of the unaltered whole blood measured by the present invention. Such unaltered whole blood includes ~~red~~ blood cells suspended in plasma which includes many things other than red blood cells.

8. For example, the plasma protein concentration greatly affects the amount of scattering by red blood cells suspended in plasma because the refractive index of plasma depends on its protein concentration. Because the amount of light scattering depends on the difference between the refractive indices of plasma and red cells, the magnitude of the light scattering in an unknown sample would be different from what it was when any sort of "correction" run occurred. Consequently, the magnitude of the light scattering estimated by the previously established "correction" would not yield a valid estimate of scattering, and all subsequent computations would be nonsense.

9. Second, different hemoglobin species in the blood affect the amount of the light absorbance and scattering. For example, if the "correction" were established with blood composed of 90% oxyhemoglobin and 10% deoxyhemoglobin, the previously established "correction" would not yield a valid measure of light scattering if the unknown sample contained 75% oxyhemoglobin because light scattering depends on the actual chemical composition of the blood.

The dependence of light scattering on the chemical composition of the blood was completely unknown to Anderson et al., whereas Figures 4 and 5 of the present application teach composition-dependence of the red blood cell scattering vector and the nonspecific scattering vector. Thus, in the case in which the chemical composition of the blood varies (in reality, a very common occurrence), the "correction" method suggested by the Examiner would completely fail.

10. Third, if chylomicrons and other lipid droplets are present in the plasma, these also scatter light, thus rendering the "correction" method suggested by the examiner completely useless. By contrast, the inclusion of chylomicrons and other lipid droplets in plasma (once again a normal occurrence), causes only minimal interference in measurements made by the present invention.

11. Fourth, if the sample used to create the "correction" had a hemoglobin concentration within the red blood cells that is different from the sample presently under consideration, the "correction" method suggested by the Examiner would fail to yield a valid estimate of light scattering by red blood cells because the refractive index of the red blood cells depends on the intracellular hemoglobin concentration. Once again, the present invention is capable of accurately determining the concentrations of a plurality of blood components despite differing intracellular hemoglobin concentration.

12. As a fifth example of the failings of the "correction" method suggested by the Examiner, consider the case of red blood

cells that differ from normal in their size and shape. Spherocytosis, osmotically swollen red blood cells, and sickle cell anemia are specific clinical conditions in which such changes occur. If the Examiner's "correction" had been established for normal blood, it would yield specious estimates of the magnitude of light scattering because light scattering by red blood cells depends on their size and shape. Again, the use of the "correction" method suggested by the Examiner would fail miserably.

13. Sixth, consider the case in which a substantial fraction of the red blood cells in the clinical sample are hemolyzed. The erroneous "correction" established in the manner suggested by the Examiner would reflect the light scattering only when all of the red blood cells in the reference blood are intact. Therefore, the Examiner's "correction" would fail to yield a valid measurement of light scattering when some unknown fraction of the red blood cells or all of the red blood cells were ruptured. Again, all subsequent computations would be grossly erroneous. By contrast, the present invention yields valid measurements both when the red blood cells in the blood samples are intact and when they are completely hemolyzed (page 40, Table IV of the present application).

14. The foregoing examples illustrate the sample-to-sample variation in light scattering that commonly occur in clinical blood samples. To anyone of ordinary skill in this technology, each single example is convincing proof that using Anderson et al., either alone or in combination with Brown et al., as the Examiner proposes, would not yield a functional device capable of measuring

the concentrations of multiple "components of unaltered whole blood" as required by independent claim 1. Furthermore, in each of the foregoing examples, using Anderson et al. as a "correction" in the ill-defined manner suggested by the Examiner would require measuring the total hemoglobin concentration by some independent method, and even then the quantity obtained would be a constant that would not reflect the true magnitude of light scattering in the blood sample at hand.

15. In contrast, the invention of the above-identified application computes concentrations of the components of whole unaltered blood of unknown composition through the use of a scattering subset of radiation wavelengths that are used to correct for the effects of radiation scattering from red blood cells and from other scattering factors existing in unaltered blood.

16. The Brown et al. reference does not supply any of the above-noted discrepancies existing in the Anderson et al. reference. In particular, the Brown et al. reference presents multi-wavelength spectrophotometry which uses one wavelength for each blood component to be determined (see Figure 5 and col. 3, lines 31-39). Thus, the only set of wavelengths contemplated by Brown et al. is a set of absorbance wavelengths, and there is no disclosure or suggestion of a set of scattering wavelengths, as required by the present invention.

17. In fact, the only scattering correction contemplated by Brown et al. is accomplished by hemolyzing the blood sample before optical measurements are made. See col. 6, lines 41-43; col. 8,

lines 2-7 and 21-22; col. 10, lines 8-9; col. 13, lines 55-68; col. 14, lines 16-17; col. 15, line 43; col. 16, lines 8 and 40; col. 17, line 6 and 37, and col. 18, lines 9 and 36-37.

18. Thus, Anderson et al. contemplate measurement of altered blood in the form of a red cell suspension which suspends nonhemolyzed red blood cells in isotonic saline, whereas Brown et al. require hemolysis and dilution of whole blood, before optical measurements. Neither Brown et al. nor Anderson et al., either alone or in combination, make measurements on whole unaltered blood, as required by the present invention.

19. In fact, if an apparatus embodying the Brown et al. invention were to have the hemolyzer disabled, completely erroneous results would occur, similar to the erroneous results depicted in the comparative example appearing in the present patent application on pages 38-41.

20. I also understand that the Examiner relies upon the teachings of Curtis et al. reference to reject claims in the present application. In my opinion, reliance upon the teachings of Curtis et al. to reject claims in this application is totally unfounded. In particular, Curtis et al., like Anderson et al. and Brown et al., require alteration of the blood sample, before optical measurements are made in order to reduce the effects of light scattering present in whole unaltered blood. In particular, Curtis hemolyzes the blood with a lysing agent which breaks up the red blood cells to release hemoglobin into solution. See col. 2, lines 4-6 and col. 5, lines 45-52.

21. In addition, Curtis does not disclose an absorbance subset of wavelengths distinct from a scattering subset of wavelengths, as required by the present invention. In particular, Curtis sought two wavelength passbands for which oxyhemoglobin and deoxyhemoglobin have approximately equal absorptivity deltas (average absorptivity at passband 1 minus average absorptivity at passband 2 being approximately the same for oxy- and deoxyhemoglobin). His goal was to measure total hemoglobin concentration using only the difference in absorbance at two wavelength passbands; therefore, he was forced to seek the same delta for oxyhemoglobin and deoxyhemoglobin so that the answer he obtained for hemoglobin concentration did not depend much on the state of oxygenation of the sample. There is absolutely no disclosure or suggestion in Curtis of a scattering subset of wavelengths of any kind, much less a scattering subset of wavelengths that have been "selected to maximize the effects of radiation scattering by unaltered whole blood relative to the effects of radiation absorbance by unaltered whole blood."

22. In conclusion, the prior art references of which I am aware, including references c-e mentioned above in paragraph 2, do not teach one of ordinary skill in this technology, and do not teach me personally, how to make and use the invention disclosed and claimed in the above-identified patent application. Further, even in view of the scope and content of the disclosure of the prior art, the invention as claimed in the present patent application, as a whole, would not have been obvious at the time

the invention was made to a person having ordinary skill in this art.

23. I hereby declare that all of the statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like are punishable by fine and/or imprisonment under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified application, or any patent issuing therefrom.

July 10, 1995
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PUBLICATIONS (in reverse chronological order)

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